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ORIGINAL ARTICLE

Improved detection of microbial ureteral stent colonisation by sonication

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Abstract

Purpose The diagnosis of microbial ureteral stent colonisation (MUSC) is difficult, since routine diagnostic techniques do not accurately detect microorganisms embedded in biofilms. New methods may improve diagnostic yield and understanding the pathophysiology of MUSC. The aim of the present study was to evaluate the potential of sonication in the detection of MUSC and to identify risk factors for device colonisation.

Methods Four hundred and eight polyurethane ureteral stents of 300 consecutive patients were prospectively evaluated. Conventional urine culture (CUC) was obtained prior to stent placement and device removal. Sonication was performed to dislodge adherent microorganisms. Data of patient sex and age, indwelling time and indication for stent placement were recorded.

Results Sonicate-fluid culture detected MUSC in 36%. Ureteral stents inserted during urinary tract infection (UTI) were more frequently colonised (59%) compared to those placed in sterile urine (26%; $P < 0.001$). Female sex ($P < 0.001$) and continuous stenting ($P < 0.005$) were significant risk factors for MUSC; a similar trend was observed in patients older than 50 years ($P = 0.16$). MUSC and indwelling time were positively correlated ($P < 0.005$). MUSC was accompanied by positive CUC in 36%. Most commonly isolated microorganisms were Coagulase-negative staphylococci (18.3%), Enterococci (17.9%) and Enterobacteriaceae (16.9%).

Conclusions Sonication is a promising approach in the diagnosis of MUSC. Significant risk factors for MUSC are UTI at the time of stent insertion, female sex, continuous stenting and indwelling time. CUC is a poor predictor of MUSC. The clinical relevance of MUSC needs further evaluation to classify isolated microorganism properly as contaminants or pathogens.

Keywords Biofilm · Microbial colonisation · Sonication · Ureteral stent · Urinary tract infection

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Introduction

Ureteral stents offer an ideal surface for microbial adherence and consecutive device colonisation. In the majority of cases, microbial ureteral stent colonisation (MUSC) remains asymptomatic. However, MUSC-related infectious complications are one of the leading serious risk factors associated with ureteral stent placement. During manipulation or instrumentation, biofilm organisms could be shed into the urine and lead to uncomplicated UTI up to urosepsis [1, 2]. The issue of MUSC has not been well studied previously. Available data are inconsistent and controversial [2–10]. Microbiologic diagnosis of MUSC is difficult, since routine culture techniques do not accurately detect microorganisms embedded in biofilms. Sonication is a short, low-energy ultrasound treatment that may improve the detection of MUSC by liberating sessile biofilm

organisms. However, most sonication devices have not been optimised for releasing pathogens from biofilms but killing pathogens susceptible to sonication. We developed a sonication system whose ultrasound power was monitored by a hydrophone and validated for optimal yield of bacteria and report the results of a prospective clinical trial using this new sonication technique for the detection of MUSC. The association between MUSC and CUC, UTI at the time of stent insertion, indwelling time, gender, single and continuous stenting as well as different indications for stent placement were investigated.

Materials and methods

Study population

All consecutive patients who had their stent removed were eligible for the study. The study has been approved by the local human subjects committee, and all included patients gave written informed consent.

Laboratory investigations

Ureteral stents were removed under aseptic conditions, divided into small parts, placed in sterile tubes and processed by the microbiology laboratory within 6 h. Negative controls ($n = 8$) consisted of unused sterile ureteral stents unpacked in the operation room and sent for sonication to go through the same process as stents removed from the patients. CUC were obtained prior to stent insertion and removal. Stent colonisation was detected by sonication as described previously [11]. In brief, 10 ml of Ringer's solution was added, tubes were vortexed for 30 s and sonicated (frequency, 40 ± 2 kHz and power density, 0.22 ± 0.04 W/cm^2, as determined by a calibrated Hydrophone, Type 8103; Brüel and Kjær, Naerum, Denmark) in an Aquasonic Model 750T ultrasound bath (VWR Scientific Products) for 1 min, followed by additional vortexing for 30 s. The resulting sonicate fluid was plated in 0.5-ml aliquots onto aerobic sheep blood agar plates (BD Diagnostic Systems) and incubated at 35–37°C in 5–7% carbon dioxide aerobically for 2 days. Microorganisms were enumerated and classified by routine microbiologic techniques.

Definitions

Single ureteral stenting

Stents obtained from patients undergoing singular stenting or the first stent removed from patients with continuous stenting.

Continuous ureteral stenting

Stents obtained from patients with continuous stenting with the exception of the first placed stent.

Positive sonicate-fluid culture (SFC)

Growth of $\geq 10^2$ CFU/ml. Since no validated cut-off value for MUSC diagnosed by sonication exists, a threshold of $\geq 10^2$ CFU/ml was chosen according to recommendations for intravascular catheters [12].

Ureteral stent contamination

Growth of $<10^2$ CFU/ml and mixed gram-positive flora in SFC.

Positive urine culture (CUC) and urine contamination

CUC were interpreted according to Wilson et al. [13] regarding collecting technique, quantitation (CFU/ml) and number of microorganisms isolated.

Statistical analysis

Analyses were performed using R (R Development Core Team 2009) [28] and the R package geepack, which implements the generalised estimating equations (GEE) approach for fitting marginal generalised linear models to clustered data [29]. A P-value of less than 0.05 was considered to indicate statistical significance; all tests were two-sided. McNemar's chi-squared test and generalised estimating equations [30] were applied as appropriate.

Results

Four hundred and thirty polyurethane stents were removed during the study period. In patients with bilateral stents ($n = 17$), only one device was considered. Four patients refused consent, in one case CUC at the time of stent removal was not submitted to the laboratory. Data of 408 stents from 300 consecutive patients fulfilled the case definition.

Sonicate-fluid culture (SFC) detected MUSC in 36%. Ninety-three discordant observational pairs were positive with sonication only, compared to eight positives with CUC only. All negative controls ($n = 8$) were negative in SFC.

UTI at the time of insertion offers a significant risk factor for MUSC (59.2%) compared to sterile urine (26%). The odds ratio, obtained from univariate GEE analysis assuming an exchangeable correlation structure,

comparing patients with and without UTI at stent insertion was 3.16 (95% confidence interval (CI) = 1.91–5.22; $P < 0.001$).

The proportion of positive SFC was lower among devices obtained from singular stenting (30%) compared to continuous stenting (49%), whereas the odds ratio comparing continuous with single ureteral stenting was 1.96 (95% CI = 1.25–3.08; $P < 0.005$).

Stents obtained from female patients showed significantly higher positive SFC proportion (55%) than stents obtained from males (24%). The crude odds ratio comparing females with males was 3.72 (95% CI = 2.35–5.89; $P < 0.001$).

Duration of stenting and rate of MUSC were positively correlated. Stents left for up to 30 days, 31–90 days and more than 90 days were colonised in 27, 39 and 54%, respectively (Table 1). The odds ratios comparing indwelling times of 31–90 days with ≤30 days and >90 days with ≤30 days were 1.67 (95% CI = 1.07–2.62; $P = 0.025$) and 2.97 (95% CI = 1.60–5.52; $P = 0.001$). In order to avoid confounding, we included UTI at stent insertion, continuous stenting, gender, age and indwelling time as ordered categorical variables in a multivariable GEE analysis (Table 2).

SFC encountered a total of 224 microorganisms (Table 3); i.e., on average 1.5 microorganisms per colonised stent. Thirty-three different organisms were identified and differentiated in nine subgroups. Coagulase-negative staphylococci spp. (CoNS) were most commonly isolated (18.3%), followed by *Enterococcus spp.* (17.9%) and Enterobacteriaceae (16.9%). SFC observed single microbial growth in 59% and multiple growths in 41%, respectively. From a total of 224 isolated microorganisms, 67% were gram-positive, while 22% were gram-negative and 11% fungi, respectively. Pure bacterial growth was found in 83%, fungal in 9% and mixed growth in 8%. The spectrum of microorganisms identified in cases with positive SFC and positive CUC correlated in 90%. In 32%, SFC revealed additional microbial growth. Most commonly isolated by CUC were Enterobacteriaceae 26%, *Enterococcus spp.* 22% and Candida spp. 17%. CUC missed MUSC in 64%.

Discussion

Biofilm formation on ureteral stents was first noted in the early 1990s by Reid et al. [10]. Despite great interest in

Table 1 Stent characteristics

Variables	No. of stents	SFC positive	SFC negative
Study group	408 (100)	145 (36)	263 (64)
Unique ureteral stent insertion	287 (70)	86 (30)	201 (70)
Continuous stent insertion	121 (30)	59 (49)	62 (51)
Sex			
Male	258 (63)	63 (24)	195 (76)
Female	150 (37)	82 (55)	68 (45)
Age			
Up to 50 years	150 (37)	46 (31)	104 (69)
>50 years	258 (63)	99 (38)	159 (62)
Indwelling time			
Up to 30 days	184 (45)	51 (28)	133 (72)
31–90 days	178 (44)	70 (39)	108 (61)
>90 days	46(11)	24 (52)	23 (48)
Indication for ureteral stent placement			
Ureterorenoscopy	122 (30)	29 (24)	93 (76)
Kidney transplantation	69 (17)	20 (29)	49 (71)
Obstructive uropathy	70 (17)	23 (33)	47 (67)
Hydronephrosis due to malignancy	26 (6)	16 (62)	10 (38)
Shock-wave lithotripsy	18 (4)	8 (44)	10 (56)
Other	103 (25)	49 (48)	54 (52)
Antibiotic prophylaxis at stent insertion	385 (94)	131 (34)	254 (66)
Urine at stent insertion	307 (75)	104 (34)	203 (66)
UTI	71 (23)	42 (59)	29 (41)
No UTI	236 (77)	62 (26)	174 (74)

Data represent numbers, percents in parentheses, *SFC* sonicate-fluid culture, *UTI* urinary tract infection

Table 2 Multivariable GEE fit of significant microbial stent colonisation using sonicate-fluid culture

Covariate	Odds ratio	95% CI		P-value
UTI at stent insertion	2.59	1.47	4.56	0.001
Continuous stenting	2.24	1.28	3.93	0.005
Female sex	3.35	1.89	5.94	<0.001
Indwelling time*				
Indwelling time[1]	1.83	1.08	3.09	0.025
Indwelling time[2]	3.02	1.37	6.66	0.006
Age >50 years	1.67	0.93	3.00	0.086

* Indwelling time ≤30 days as reference

[1] Indwelling time 31–90 days

[2] Indwelling time >90 days

Table 3 Microorganisms detected by sonicate-fluid culture

Microorganism	Total no.		Male sex		Female sex	
No. of microorganisms	224	R	93	R	131	R
CoNS[1]	41 (18.3)	1	25 (26.9)	1	16 (12.2)	3
Enterococcus spp.	40 (17.9)	2	19 (20.4)	2	21 (16)	2
Enterobacteriaceae[2]	38 (16.9)	3	12 (12.9)	3	26 (19.8)	1
Candida spp.	24 (10.7)	4	11 (11.8)	4	13 (9.9)	5
Lactobacillus spp.	19 (8.5)	5	2 (2.2)	7	17 (13)	4
Streptococcus spp.	18 (8)	6	6 (6.5)	5	12 (9.2)	6
Corynebacterium spp.	12 (5.4)	7	4 (4.3)	6	8 (6.1)	7
Gardnerella vaginalis	6 (2.7)	8	1(1.1)	8	5 (3.8)	8
Other[3]	26 (11.6)	9	13 (14)	9	13 (9.9)	9

R (rank), data represent numbers, percents in parentheses

[1] Coagulase-negative staphylococci spp

[2] *Escherichia coli* ($n = 27$), *Serratia marcescens* ($n = 2$), *Citrobacter freundii* ($n = 1$), *Proteus mirabilis/peneri* ($n = 3$), *Klebsiella pneumoniae* ($n = 2$), *Enterobacter cloacae* ($n = 1$), *Enterobacter aerogenes* ($n = 1$)

[3] *Kocuria rosea* ($n = 5$), *Kocuriae kristinae* ($n = 3$), *Dermacoccus nishinomiyaensis* ($n = 1$), *Staphylococcus aureus* ($n = 8$), *Pseudomonas aeruginosa* ($n = 4$), *Erysipelothrix rhusiopathiae* ($n = 1$), *Actinobaculum Schaalii* ($n = 1$), *Moraxella catarrhalis* ($n = 1$), Gram-labile rods ($n = 2$)

clinical-related biofilm research to date, only a few studies conducted further investigations in this field [2–10]. Available data about MUSC are inconsistent and controversial.

In the majority of previous studies, a detailed description regarding the microbiological diagnostic technique is missing. Kehinde et al. [8] used the semiquantitative role and plate method and observed MUSC in 42%. Klis et al. [9] reported about MUSC in 98.5% detected by qualitative broth culture. However, these two diagnostic techniques have limitations like the risk of contamination [14]. Furthermore, qualitative broth culture cannot quantify the number of microorganisms; thus, it is not possible to differentiate between MUSC and contamination. A further disadvantage of the role and plate method is that it allows detection of outer surface bacteria only.

Sonication is a short, low-energy ultrasound treatment that improves the diagnosis of microbial medical device colonisation by liberating organisms encased in biofilms [11, 15–17]. Technically, ultrasound waves radiate through a solution and produce high- and low-pressure areas. During the low-pressure stage, millions of microscopic vapour bubbles are formed, which collapse during the high-pressure stage releasing an enormous amount of energy on the surface of the objects. This agitation causes a vacuum-scrubbing action by releasing acoustic energy at the surface of objects [18, 19]. We predominantly detected high numbers of organisms (at least 10^2 CFU per plate). Quantification of the number of microorganisms and detection of polymicrobial growth is another advantage of sonication. Although trials have evaluated the potential of sonication in diagnosing MUSC previously [4, 7, 10], the results of these studies have to be taken with precaution, since they bear methodological flaws. Ultrasound is defined by frequencies above the range of human hearing (20 kHz). Two studies [4, 7] used frequencies of 50 Hz, and in one study, technical details of sonication were missing [10]. In addition, biofilm removal by sonication strongly depends on the intensity of sonication energy (power density) and to a far lesser extent on frequency [20]. Inappropriate power density may lead to elimination of microorganisms due to thinning of cell membranes, localised heating and production of free radicals [21]. Gram-negative bacteria were more susceptible to these detrimental effects than gram-positives due to the lack of a thick and robust cell wall. The relative high proportion of gram-positive microorganisms detected in previous studies using sonication [4, 7, 10] may be attributed to the elimination of gram-negative bacteria by ultrasound due to inappropriate power densities. Therefore, measuring power density by a calibrated hydrophone should be an obligatory step prior to using sonication. A disadvantage of sonication is the potential release of biofilm organism that might not be clinically relevant.

The role of CUC in predicting MUSC was discussed previously [2–8]. However, an exact differentiation between pathogens and contaminating organisms is missing in all studies, resulting in a higher rate of positive CUC when compared to our results. We could confirm the reported impaired value of CUC in predicting MUSC. However, this is not astonishing since CUC can only detect free floating. Positive CUC was missing in about 60% of colonised stents. Since MUSC does not necessarily lead to bacteriuria, negative CUC does not rule out stent colonisation. The higher prevalence of MUSC on devices

obtained from continuous stenting compared to single stenting is explainable with the replacement technique via a guide wire. The wire may displace intraluminal biofilm fragments, and the new device could be inoculated as it slides over. The rate of MUSC was significantly higher in stents removed from females compared to those from males. Since UTI and bacteriuria are more common in women than in men, this observation is comprehensible. Previous studies showed similar results [2–4, 8]. The increase in MUSC in stents derived from patients older than 50 years might be associated with the age-related increase in bacteriuria. In accordance with previous studies [2, 3, 7], we observed a positive correlation between indwelling time and rate of MUSC.

We predominantly detected CoNS, *Enterococci spp*. and Enterobactericeae by SFC. The variation of isolated microorganisms compared to other studies may be explainable with variations in the spectrum of different hospitals and countries [8], the diagnostic technique and the study population. CoNS have long been dismissed as culture contaminants. Today, they are recognised as one of the most frequent causes of biofilm-associated infections [22, 23]. CoNS frequently colonise skin and mucus surfaces. Any medical device that penetrates those surfaces during surgery is at high risk to become colonised. The high prevalence of CoNS in the distal male urethra [24] may be responsible for the frequent detection of these organisms on stents obtained from male patients. *Enterococcus spp.* have been associated with biofilms on various kinds of indwelling medical devices [25]. In the present trial, *Escherichia coli* was the most common gram-negative organism isolated by SFC. This gram-negative rod uses several virulence factors like fimbrial protein fimH. Fimbrial protein binds to Tamm-Horsfall protein (THP). THP has been found to adhere to ureteral stents [26] and therefore may act as a promoter of MUSC [27].

How could our findings be transferred into clinical practice? Although during stent manipulation or instrumentation, undetected biofilm organisms may be shed into the urine, serious infectious complications [1, 2] are rare. Thus, we could not recommend sonication as a standard procedure in every patient. However, once a serious biofilm-associated infectious complication occurs, the attending clinician might be surprised by his microbiological opponents. Microbiological investigations like blood and urine cultures would be initiated. But the quantity, aggressiveness and susceptibility pattern of the responsible pathogen remain unknown for at least 24–48 h and initial antimicrobial treatment is empiric. Patients especially with a high risk of infectious complications (e.g. patients with immunosuppressants, diabetes mellitus or artificial cardiac valve) would benefit from an early identification of biofilm organisms by sonication.

Reid et al. [10] posed the question how important is MUSC in asymptomatic patients? We would add the question, how important is MUSC in symptomatic patients? Is it possible that patients with an indwelling ureteral stent develop symptoms due to MUSC? A prospective clinical trial analysing the relationship between MUSC, LUTS and other stent-related symptoms could elucidate this issue. Moreover, these data might improve pharmaceutical treatment and the development of appropriate coatings to delay stent colonisation.

Some limitations of this study should be mentioned: (1) The current lack of a gold standard definition of MUSC-related infection (2) No validated microcobiological gold standard technique exist for the diagnosis of MUSC (3) The cut-off value of SFC for MUSC was chosen according to a reference for intravascular catheters (4) Only conventional microbiologic culture techniques were applied to identify microbial growth. Novel and more sensitive approaches like immunfluorescence microscopy as well as amplification of the 16S rRNA may be more sensitive to detect fastidious organisms.

Conclusion

The results of our study demonstrate that sonication is a promising approach in the diagnosis of MUSC. Results of CUC are a poor predictor of MUSC. Stents placed during UTI and devices derived from females, patients older than 50 years and continuous stenting are at major risk to be colonised. The clinical relevance of MUSC needs further evaluation to classify isolated microorganism properly as contaminants or pathogens.

Conflict of interest statement There is no conflict of interest.

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